

MAPLE SIRUP. XI. RELATIONSHIP BETWEEN THE TYPE AND
ORIGIN OF REDUCING SUGARS IN SAP AND THE
COLOR AND FLAVOR OF MAPLE SIRUP ^a

Maple sirup made from early, cold-weather sap flows or from sterile sap has a light amber color and a delicate flavor. Sirup made from late-season or from fermented sap usually possesses a flavor denoted as caramel and also has a dark amber or brown color which decreases its value (2, 3, 6, 7). Analyses of sirups have shown that with increasing coloration and caramel flavor there is also an increase in concentration of reducing sugars (invert) (2, 10). Fresh, sterile maple sap contains no hexose sugars (4, 8), but fermentation of sap with certain microorganisms results in sirup containing invert sugar and caramel (5). It has also been shown, however, that under certain controlled conditions, fermentation of sap results in enhancement of the maple flavor without the production of caramel.

This paper reports studies made to determine the effect of the type and origin of reducing sugars in sap on the formation of color and flavor in maple sirup. To determine their possible specificity in producing these color and flavor changes, the following reducing sugars were added to sterile sap before concentrating it to sirup: simulated invert obtained from a mixture of crystalline glucose and fructose; crystalline maltose; reducing sugars derived from crystalline sucrose and maltose by enzymic and ion-exchange resin hydrolysis; and reducing sugars from carbohydrates in sap by the action of invertase, which was added to the sterile sap 4 days before concentrating it to sirup.

MATERIALS, METHODS, AND PROCEDURE

Materials. Maple sap was collected under aseptic conditions using the technique previously described (7). The sap from each sterile 5-gallon collecting bottle was aseptically dispensed into sterile 1-gallon metal cans, quick-frozen, and stored at -29°C . The sterility of the collected sap was determined by sampling each collecting bottle and plating the samples in nutrient agar. Any lot of sap having a count exceeding 1 colony per milliliter was discarded. Since only 4 portions of sap could be conveniently reduced to sirup in 1 day, the experiment was carried out at 4 different times with 4 lots of sap. Careful selection of sap was required so that identical replicates could be used throughout the 4 parts of the study. To obtain this uniformity, eight 4-gallon collections of sterile sap were selected. One gallon of sap from each collection was allocated to each of the 4 lots so that at the completion of the allocation each 8-gallon lot contained equal volumes of sap from the same trees and the same flows.

To prepare each lot of frozen sap for use, a procedure was developed to thaw the sap rapidly in a manner which minimized microbial contamination and chemical changes. The desired amount of frozen sap was removed from the gallon cans, crushed in a

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sanitized power crusher and melted rapidly in a steam-jacketed kettle. To prevent changes in the sap during heating, the kettle was charged with about 1 gallon of the crushed frozen sap, heat was applied by steam at 5 p.s.i., and the ice was slurried rapidly until most of it had melted, at which time the sap was removed. In a short time the residual ice melted, the final temperature of the sap being 15°-20° C. The melted sap was blended and divided into 4 portions of 2 gallons each.

The carbohydrates were added to the sap at a concentration of 1 g. per 2 gal. The reducing sugars evaluated in this study consisted of the following:

- a. Simulated invert was prepared by combining equal weights of crystalline glucose and fructose.
- b. Invert sugar was prepared from sucrose by 3 methods. These methods of inverting sucrose were investigated to determine if they produced cleavage products with different or peculiar reactivities, even though of a transitory nature.
 1. Enzyme inversion. A 10% solution of sucrose was treated with invertase (0.2 ml. of Convertit[°] per 10 ml. of solution) for 2 hours at 15° C.
 2. Ion-exchange inversion. A 4% solution of sucrose was treated with Dowex-50 resin[°] in the H-ion phase. Fifty milliliters of sucrose solution was heated with 10 g. of Dowex-50 for 10 min. at 65-70° C. The mixture was filtered to remove the resin and washed to a volume of 100 ml. A 50-ml. aliquot (equivalent to 1 g. of carbohydrate) was added to the sap immediately before reduction to sirup.
 3. Inversion of sucrose of maple sap. A 2-gallon portion of sap was treated with 0.2 ml. of Convertit[°] and incubated for 4 days at about 1° C.
- c. Maltose was used as a representative reducing disaccharide.
- d. Hydrolyzed maltose was prepared by treating with Dowex-50 resin in the H-ion phase. Fifty milliliters of a 4% solution of maltose was treated with 10 g. of Dowex-50 for 2 hours at 65-70° C. The mixture was filtered to remove the resin, washed to 100 ml. and a 50-ml. aliquot was added to the sap immediately before reduction to sirup.

Methods. Refractive index measurements for determining per cent solids as sucrose (degrees Brix) were made at 20° C. with an Abbe refractometer. Color indices^d were

[°] Mention of trade name does not imply endorsement of the U. S. Department of Agriculture over similar products not mentioned.

^d Color index, $A_{1\text{ cm}}^{86.3\%} = A_{450} (86.3/bc)$, where A_{450} is the observed absorbance at 450 mμ, b is the depth of solution in centimeters and c is the grams of solids as sucrose per 100 ml.

TABLE 1
Arrangement of experimental treatments and controls among the four lots of sap

Portion	Lot A	Lot B	Lot C	Lot D
1	Untreated control	Sucrose	With invertase for 4 days ¹ at about 1° C.	Maltose hydrolyzed with Dowex-50
2	Sucrose inverted with invertase	Untreated control	Simulated invert	Sucrose inverted with invertase
3	Inactivated invertase	Maltose	Untreated control	With invertase for 4 days ¹ at about 1° C.
4	Simulated invert	Sucrose inverted with Dowex-50	Sucrose inverted with invertase	Untreated control

¹ The sap in this instance was reduced to sirup 4 days later than other portions from this lot.

determined from absorption measurements at 450 m μ made on sirups of 65–67° Brix filtered with pressure through three layers of Whatman[®] No. 52 filter paper in a Seitz filter. Per cent of invert sugar was determined by the Berlin method (1). Flavor evaluations of the sirups were made by an experienced panel.

Procedure. An 8-gallon lot of frozen sap was thawed for each of the 4 parts of the study. The melted sap was divided into 2-gallon portions and treated as shown in Table 1. This randomized distribution was selected to avoid any differences that might have been caused by variations in daily or within-day operations. One portion from each lot was an untreated control, while reagent controls were provided by one portion that was treated with 1 g. of sucrose (B1) and one with 0.2 ml. of inactivated invertase (A3).

The 2-gallon portions of sap were reduced to sirup under standardized conditions selected to simulate conditions occurring in a commercial evaporator. In a steam-jacketed kettle, sap was evaporated rapidly (30–35 minutes) to a volume calculated to give a density of approximately 45° Brix. This sirup was transferred to a stainless steel beaker equipped with a condenser and refluxed for one hour over a Meeker burner. The refluxed sirup was transferred to a 1-quart steam kettle and evaporated rapidly (5–8 minutes) to standard density sirup (65.5° Brix).

Determinations were made on the sirups prepared from treated sap as follows: per cent of invert sugar, color indices, and flavor evaluations.

RESULTS AND DISCUSSION

Effects of adding reducing sugars to maple sap on the color and flavor of the sirup are summarized in Table 2. The close agreement in values obtained for reducing sugars calculated as invert sugar and for color index in both the untreated and reagent control samples indicates that the empirical procedure devised is reproducible from day to day and that addition of the reagents did not affect the flavor, color, or invert content of the sirup produced.

Adding reducing sugars derived from sucrose either by enzyme inversion, by ion-exchange hydrolysis, or by simulation with equal parts of glucose and

TABLE 2
Effect of type and source of reducing sugar on color and flavor of maple sirup

Lot— Portion	Treatment	Invert sugar	Color index	Degree of caramel ¹
		%		
A1	Untreated control.....	0.15	0.51	-----
B2	Untreated control.....	0.16	0.51	-----
C3	Untreated control.....	0.16	0.52	-----
D4	Untreated control.....	0.18	0.56	-----
B1	Sucrose.....	0.16	0.51	-----
A3	Inactivated invertase.....	0.16	0.50	-----
C2	Simulated invert.....	0.55	0.75	+
A4	Simulated invert.....	0.81	0.92	++
A2	Sucrose inverted with invertase.....	0.72	0.91	++
C4	Sucrose inverted with invertase.....	0.80	0.77	++
D2	Sucrose inverted with invertase.....	0.74	0.98	++
B4	Sucrose inverted with Dowex-50.....	0.41	0.72	++
C1	Sap treated with invertase for 4 days at 33-34° F.....	53.3	3.25	++++
D3	Sap treated with invertase for 4 days at 33-34° F.....	56.3	2.90	++++
B3	Maltose.....	0.56	0.77	++
D1	Maltose hydrolyzed with Dowex-50.....	0.49	0.87	+

¹ Indicates no caramel detected; + to ++++ indicates increasing degrees of caramel.

fructose resulted in the production of appreciable color and caramel flavor.* When sap was treated with invertase for 4 days at about 1° C., resulting sirup had an exceedingly dark color and strong caramel flavor. Under the conditions used over half of the sucrose present was inverted.

Maltose was chosen as an example of a reducing oligosaccharide which upon hydrolysis would yield glucose only. Use of this carbohydrate and its hydrolytic products also resulted in increased color and the production of caramel flavor.

Addition of reducing sugars did not seem to increase the level of maple flavor, although it is quite possible that the presence of caramel obscured any maple flavor that might have been formed. Development of color and caramel flavor from sap containing invert sugar is consistent with facts observed under practical conditions of maple sirup production. In most cases the darker grades of sirup are characterized by stronger caramel flavor and an increasing content of invert sugar (2, 10). Such sirups are produced under conditions favoring microbial growth, and the reducing sugars must be the product of sucrose inversion. It has been shown that sterile sap contains no measurable amount of hexoses and only a trace of oligosaccharides which could be reducing (8).

Recently, Underwood, Lento and Willits (9) have demonstrated the presence of trioses in maple sirup. This offers a possible mechanism whereby reducing sugars give rise to the development of color and flavor. However, this does not fully explain the enhancement of maple flavor following the controlled fermentation of sap with a *Pseudomonas* sp. (5). The possible role of other products of the fermentation is strongly suggested.

* This is the sharp pungent flavor so characteristic of dark sirups.

SUMMARY

Incorporation of reducing sugars in maple sap before reduction to sirup by atmospheric boiling results in increased color and caramel flavor irrespective of the source of the sugars. Although there was no detectable enhancement of maple flavor, this could have been obscured by the presence of caramel.

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